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## THE DIAGNOSTIC VALUE OF THE DIPH- THERITIC BACILLUS.\*

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In the second volume of the *Mittheilungen aus dem Kaiserlichen Gesundheitsamte*, published in 1884, there appeared a contribution by Loeffler on the bacterial nature of the disease called diphtheria. Loeffler states in this paper that he has isolated in pure cultivations the organism described by Klebs the year before as occurring in the diphtheritic membrane and considered by him as the cause of the disease, and that he has succeeded in reproducing from inoculations of his pure cultures into susceptible animals a disease possessing anatomical characters identical in all essential particulars with human diphtheria.

At the time of Loeffler's first communication he expressed himself with some reserve on the final point whether the organism which he isolated could be considered to be the causative agency in the production of the disease diphtheria. But in the time which has elapsed

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since then one after another of the objections to the acceptance of this organism as the cause of primary diphtheria have been removed, and to-day we have, as I believe, in the bacillus of Klebs-Loeffler the true cause of primary diphtheria.

As Loeffler pointed out, it becomes necessary in the first place to distinguish between diphtheria and diphtheritis. He describes the first term as indicating "a characteristic and distinct disease due to a specific *ens morbi*, like measles and smallpox, which has continued constant for centuries, and occurs epidemically;" and by the latter term, diphtheritis, he understands exclusively "a definite pathologico-anatomical form of tissue change, which occurs in diphtheria along with other tissue changes."

In his first paper he gives the evidence on which he bases his belief that he has isolated the bacillus diphtheriae. He says: "They have been found in thirteen (13) typical cases of diphtheria, with fibrinous exudation on the fauces and in a constantly recurring arrangement; they lie in the oldest part of the membrane, and penetrate deeper than any other bacteria. Cultivations of the organism introduced beneath the skin of guinea-pigs and small birds kill them, producing a whitish or hemor-

rhagic exudation and extensive edema at the point of inoculation.

"The internal organs are not affected, as is the case with man; introduced through a wound in the trachea in rabbits, fowls, and pigeons, the poison produces a false membrane, and also if placed on the scarified connective tissue of the rabbit and in the entrance of the dilated vagina of guinea-pigs. In addition to the formation of a false membrane there has been observed the characteristic serious alteration of the vascular walls, which shows itself by bloody edema, hemorrhage into the tissues of the lymphatic glands and effusion into the pleural cavity.

"The bacilli have, therefore, the same effect as the diphtheritic virus. They also have the property in common with the virus, that they kill young animals more easily and quickly than old ones. On the other hand, the bacilli were not present in a number of undoubted cases of diphtheria. They were not found in the false membrane in rabbits and fowls arranged in the same typical manner as in man. When applied to the uninjured mucus membrane of the fauces, respiratory passages, eyes, and vagina, no effect was produced in several animals otherwise susceptible to their action.

"Animals which survived inoculation showed no paralytic symptoms. A bacterium is sometimes found in the saliva of the healthy child which morphologically and physiologically is indistinguishable from the bacillus of diphtheria."

Hence he concludes that if the bacillus is not demonstrable as the cause of diphtheria it is not excluded from so being. This was in 1884. As stated before, the doubtful cases and conditions have been cleared away one by one until, in 1890, Loeffler presented, in the *Deutsche Med. Wochenschrift*, another communication, in which he reviewed the entire subject, and from the numerous contributions from all parts of the enlightened world the conclusion is reached that the bacillus diphtheriae is the sole cause of primary diphtheria. At this time there was but one discordant series of experiments. This was that of Prudden in this country, and it was predicted by Loeffler that the doubt arising from this investigator's observations would be cleared up in time, as he did not believe that a different form of diphtheria prevailed in this country from that occurring elsewhere.

In a paper published by Drs. Welch and Abbott, in the Johns Hopkins Hospital Bulletin for 1891, this remaining doubt was re-

moved; for in a number of cases of primary diphtheria occurring in Baltimore, Md., which they examined they found the Klebs-Loeffler bacilli in every one, isolated them in pure culture, and tested their virulence on guinea pigs. Finally, Prudden himself has, in a recent number of the New York Medical Journal, published a result of a new series of experiments in which he found the bacilli of diphtheria regularly in a number of undoubted cases of the disease, and he expressed himself as convinced of their causative relation to the disease.

So it will be seen that in the bacillus of Klebs-Loeffler we have an organism that is found regularly by all competent observers in different parts of the world in undoubted cases diphtheria. That by means of this organism the disease can be reproduced in all of its essential and distinguishing features in the lower animals, and from these animals it is possible to obtain the bacilli again in pure cultivations.

If the discovery of the bacillus *diphtheriae* has shown us that diphtheria is produced by a living organism, it has enabled us also to answer other very important questions concerning the etiology of this disease. In a recent address delivered by Dr. W. H. Welch before

the Medical and Chirurgical Faculty of Maryland (Med. News, May 16, 1891), the author, in considering the light which has been shed upon diphtheria by the discovery of its specific cause, reviews the still much disputed questions concerning the disease: "Is diphtheria primarily local or constitutional in its organism? Are all pseudo-membranous inflammations of the throat, not directly referable to caustic irritants, diphtheria? Is there a purely local, non-contagious, pseudo-membranous laryngitis called croup distinguishable from diphtheria? Are the pseudo-membranous anginas secondary to scarlatina, and less frequently to measles and some other infectious diseases, identical with diphtheria? Is there any relation between follicular tonsillitis and diphtheria? May diphtheria occur in a mild form as a simple catarrhal inflammation of the throat? Are pneumonia, acute nephritis, suppurations of the glands of the neck, etc., referable to the direct action of the diphtheritic virus; in other words, what lesions belong directly to the disease and what are complications? Shall reliance be placed upon local or general treatment?"

To attempt an answer, as far as it is possible to do so, to all of these questions, important though they are, would extend this paper much

beyond the time it should observe. But a few of them are of such great importance that we must examine them for a moment.

One of the greatest achievements of the discovery of the diphtheritic bacillus is the proof that the virus develops locally at the site of inoculation only; that it never invades the blood and organs of the body, and that it is not capable of penetrating into the mucous membrane of the part affected. But if the bacilli develop locally they are not prevented from producing a poison which enters the blood and tissues and is capable of giving rise to those grave constitutional symptoms that are familiar to all. So, if the poison is produced locally, its effects are felt over the entire organism.

The contribution of Oertel, made in 1888, on the pathology of epidemic diphtheria, in which he described the peculiar form of cell-death found in the affected parts of the throat, in the neighboring lymphatic glands, in the spleen, Peyer's patches, and mesenteric glands, had indicated such an action of the virus. In a series of experiments on rabbits, guinea-pigs, and kittens, made by Prof. Welch and myself in the pathological laboratory of Johns Hopkins University during the past year, we have been able to confirm and extend the observa-

tions of Oertel in human diphtheria, and we have described lesions in the seat of inoculation, contiguous lymph glands, as well as those in the most remote parts of the body, in the spleen, kidneys, adrenals, intestinal epithelium, and lymphatic apparatus, in the liver, lungs, and heart, and we have, in common with other recent investigators, produced in rabbits and kittens diphtheritic paralysis.

The soluble poison of diphtheria has been isolated in a state approaching purity in recent times, and its properties have been studied somewhat by Roux and Yersin and Fraenkel and Brieger. As might have been anticipated, it has been found to be of peculiar potency, and its mode of action is so new and novel that it has opened up entirely new fields of research, and it is probable will introduce new conceptions into the subject of the action of chemicals on the animal organism.

As illustrating the intensity of the poison is the experiment of Roux and Yersin, in which they were able, by the use of 0.4 milligram of the substance obtained by evaporating to dryness, under proper precautions, the active culture fluid, to kill at least eight guinea-pigs, weighing each four hundred grams, or two rabbits, weighing each three kilograms. This

poison is capable of producing in susceptible animals all of the local and constitutional effects of the bacilli save the pseudo-membrane; for the production of the latter the bacilli are necessary.

As interesting as these facts concerning the soluble poison are, it is the peculiar and most extraordinary property of this poison that when introduced in a proper fatal dose into animals it may cause no apparent effects for days, and the death of the animal may be delayed for days, weeks, and even months. This is a new quality in a chemical poison, and one of the greatest significance in the present instance, for it is clear that although the membrane be destroyed early in the disease the individual may still die of the effect of the poison.

The chemical nature of this remarkable compound is not definitely known. It probably belongs to the albumens, yet it differs from our usual definitions of such compounds; it is non-crystallizable, and it has been called for the present the tox-albumen of diphtheria.

Enough has been said to show that diphtheria is both a local and a constitutional disease; that the primary lesion is a local one, and that the constitutional effects are secondary. But they are secondary only in time; in their importance

on the bearing of the disease they are of the greatest moment and demand the most careful attention.

Concerning the existence of pseudo-membranous anginas not diphtheria, it is now quite certain that they do occur. In scarlatina, measles, and some other infectious diseases the pseudo-membranous anginas are often different from diphtheria. These false membranes may be of bacterial origin, and in the scarlatinal pseudo-membranous angina a streptococcus is regarded as the exciting cause. And it is important to consider that in certain cases the pseudo-membranous anginas succeeding scarlet fever are diphtheria, and that scarlet fever appears to be a predisposing cause in the development of diphtheria.

There are pseudo-membranous anginas which occur independently of the acute infectious diseases, which may or may not be regarded as diphtheria at the onset. And it is recognized that diphtheria varies so greatly in its virulence that in this class of cases it is of the greatest importance to settle definitely their character. For on this knowledge will depend not only the treatment of the case under observation, but what is more important perhaps, the measures that will be taken to prevent the

spread of the disease to others in the same household and community.

The bacillus diphtheriae I believe to be the true and only cause of diphtheria, and I hope to show that the discovery of it and the study of its morphology, biology, and physiology has not only enabled us to separate the disease diphtheria from other affections simulating it in appearance, but has provided us with a definite means of diagnosis and has taught us much concerning the prophylaxis of the disease. Nor is this all; it has given us hints and direction that must lead to the use of wiser and more effective measures of treatment.

The examination of a case of suspected diphtheria for the purpose of diagnosis, while not especially difficult, presupposes a slight acquaintance at least with modern bacteriological methods and the possession of a modest bacteriological outfit and a microscope of sufficient power. Notwithstanding Roux and Yersin have endeavored to popularize the method of such examinations, it is doubtful if the practitioner at the present time possesses the means and the knowledge to carry out the requirements; but as the necessity for bacteriological training is recognized more and more by the profession, opportunities will be afforded the student in medicine to become familiar with them.

The bacillus diphtheriae is non-motile. It is about the length of a tubercle bacillus and two or three times as broad. Its morphology is among its most striking characteristics, and often renders its identification a comparatively easy matter, even on cover slips made directly from the false membrane when associated with numerous other bacteria. It appears variously, "sometimes as a regular straight or slightly bent rod, with rounded ends; it is especially characteristic to find irregular and often bizarre forms, such as rods, with one or both ends swollen, and very frequently rods broken at irregular intervals into short, sharply-marked segments, with either round, oval or straight sides. Some forms stain uniformly, others in various irregular ways, the most common being the appearance of deeply-stained granules in a lightly stained bacillus." (Welch and Abbott.)

Cover-slips are stained with Loeffler's methylene blue solution. It is, however, the culture method that serves most effectually for identification of the bacillus. The bacilli grow on blood serum, nutrient agar and gelatine, bouillon, and even on steamed potato. They also find suitable conditions for their multiplication in milk.

The best medium for their growth is the

blood-serum-bouillon mixture of Loeffler, on which they increase with great rapidity. This medium is prepared by mixing three parts of blood serum from an animal, one part of bouillon containing one per cent peptone, one per cent grape sugar, and 0.5 per cent chloride of sodium, sterilizing and solidifying. Next to this medium they grow best on nutrient agar-agar containing four per cent to six per cent of glycerine.

In preparing tubes from the false membrane it is only necessary to introduce a sterilized platinum needle into the membrane and draw it over the surface of the culture medium. Several tubes are made in this way, and a little of the exudation is rubbed on cover-glasses. The latter are examined at once, while the tubes are placed in the breeding-oven.

The bacilli do not multiply, or only very slowly, below 64° Fahrenheit; they increase rapidly under favorable conditions at the temperature of the body. They are killed by an exposure to a temperature of 136.4° Fahrenheit for ten minutes.

The cover-slips from the false membrane may show at once that the bacilli are present, but the cultures on blood serum will show unmistakably the next day. The bacilli of diph-

theria multiply with such rapidity on this medium that at the end of twenty-four hours a decided growth is found, while other bacteria are often held in temporary abeyance. Cover-slips made from this growth are then examined, and if the case was one of diphtheria the peculiar bacilli are readily made out.

To follow further the identification of the bacilli, plate cultures are made on glycerine agar. The colonies under a low power ( $\frac{1}{2}$  in.) of the microscope are quite characteristic; and finally, if it is desired, their virulence may be tested on a guinea-pig or kitten.

Up to the present part of my paper I have endeavored to show that diphtheria is a specific disease, that it is the result of the development in the part primarily affected of a particular organism—the bacillus of Klebs-Loeffler; that this bacillus has been isolated in pure cultivations, and that it is capable of reproducing in animals experimentally the natural disease found in human beings. I have also pointed out that the morphological and biological properties of it are sufficiently understood to permit of its dilution in the part affected; that it is only found locally in the seat of inoculation, and that it is as characteristic of diphtheria as the tubercle bacillus is of tuberculosis, and that

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in a similar way it may be used as a means of diagnosis. I have alluded to the light which the detection, isolation, and study of this bacillus has thrown on the prophylaxis of the disease and the suggestions it has given for the treatment of it. With a brief reference to these aspects of the subject I will close my paper.

In an article contributed by Loeffler to the *Berliner Klin. Wochenschrift*, in 1890 (*Welche Maasregeln erscheinen gegen die Verbreitung der Diphtherie geboten?*), he considers the measures which should be carried out in securing prophylaxis in diphtheria, and his conclusions are:

1. The cause of diphtheria is the bacillus diphtheriae, and it is found in the exudation of the diseased mucous membrane.
2. The bacilli are thrown off with the membrane. They can be deposited on every thing in the neighborhood of the diseased.
3. The bacilli are capable of causing infection in others as long as the slightest trace of membrane is still present, as well as for a number of days after the disappearance of the membrane.
4. Those sick of diphtheria are to be carefully isolated and kept in isolation as long as bacilli are found in the secretions. Children

who have had the disease should be kept from school not less than four weeks.

5. The diphtheritic bacilli retain their vitality in pieces of membrane for four or five months. It is therefore necessary to treat every thing that may have been infected by the patient, such as wash, bed-clothes, glasses, dishes, cloths, etc., with boiling water or live steam, while the room in which the sick has lain must be carefully disinfected. The floors are to be washed with a warm solution of bichloride of mercury, 1 to 1,000, and the walls and furniture are to be rubbed down with bread.

6. Investigations concerning the vitality of the bacilli in damp surroundings are not yet completed. They are probably more resistant under these conditions. Damp and dark homes seem to be favorable for the preservation of the vitality of the diphtheritic virus, hence such homes have to be emptied and opened for the purpose of drying them and for the entrance of light and air. In charge of places of living it is especially important that a careful disinfection of the infected home and its contents be made.

7. The bacilli increase outside of the body at  $64^{\circ}$  Fahrenheit. Milk is an excellent medium

for their multiplication. Great care is necessary not to use milk that may come from dairies in which diphtheria is prevailing.

8. Diphtheria of many animals — pigeons, hens, calves, and pigs, is not produced by the same germ that causes the human disease. These animals are not to be feared as sources of human diphtheria. Nothing positive can be said at this time of the diphtheria of cats.

9. Lesions of the mucous membrane favor the invasion of the virus. Susceptible individuals may become affected without such previous lesion.

10. In times when diphtheria prevails it is of importance to have the mouths, noses, and throats of children clean. For this purpose weak sublimate (1 to 10,000) or an aromatic wash is to be recommended.

The last communication by Loeffler to this matter has for its subject the therapeutics of the disease (*Zur Therapie der Diphtherie, Deutsche Med. Wochenschrift*, 1891, No. 10.) In the experimental examination of various drugs and agents which have been used or promised good results in the treatment of the disease, he has endeavored from the beginning of his inquiry to so conduct his work that the practical problem should be approximated as closely as possible and a practically useful result be obtained.

In combating the diphtheritic bacilli there are two points to be overcome:

1. To prevent the settlement of the bacilli on the intact mucous membrane of well persons and on the adjacent unaffected mucous membrane of those suffering from the disease. This settlement of the bacilli is to be prevented either by applying to the healthy mucous membrane such substances as hinder the development of the bacilli, or what is better, perhaps, by destroying in the shortest time possible the somewhat non resistant bacilli which have settled there. It is evident that the means must be such as will not injure the mucous membrane itself or affect the body by its poisonous properties.

2. The bacilli in the pseudo-membrane must be killed in order to prevent the spread of the disease in the person already affected and to remove the danger of transmission to others.

For the proper investigation of these features it was necessary to use a culture medium in which the bacilli grow as rapidly as in the throats of children, one which is easily and perfectly capable of observation and that admits of being maintained at the proper temperature. By the use of the blood serum-bouillon medium Loeffler believed he had secured these requirements.

His method was to inoculate such culture-tubes with a dilution in water of the bacilli by drawing a platinum needle carrying a minute quantity of the suspension of the bacilli over the surface of the solidified serum. Placed in the breeding-oven they showed a uniform coating of colonies after twenty-four hours. Into these tubes of fresh colonies, representing the bacilli in contact with the healthy mucous membrane, the reagent was brought and the contact allowed varied from momentary (the fluid being poured off immediately) to 10, 20, or 30 seconds, corresponding to the length of time one can gargle with comfort.

As soon as the reagent was removed a fresh transplantation of the colonies treated was made and the results watched and noted.

If the colonies of the original tubes inoculated with the suspension of bacilli are permitted to grow for several days, a layer of colonies about  $\frac{1}{2}$  mm. thick is obtained. This represents the growth in the superficial portions of the mucous membrane. Tubes prepared in this way were tested also, and a large number of reagents were employed. I will give one case as an example of Loeffler's method, and then his conclusions:

A solution of corrosive sublimate of the

strength of 1 to 10,000 by momentary contact would destroy the fresh culture (twenty-four hours old); with a dilution of 1 to 20,000 only a few colonies remained; but after twenty-four hours longer the growth remaining after treatment with 1 to 20,000 developed into strong colonies. Essentially weaker was the effect of a 1 to 10,000 solution on the older cultures. A solution of 1 to 2,000 with a contact of twenty seconds had not penetrated the deeper layers; but a similar contact with a 1 to 1,000 solution killed nearly all in the deeper layers. Stronger solutions killed all colonies. Cyanide of mercury proved effective, and has less of the metallic taste. Carabolic acid was satisfactory also.

Hence, in conclusion, Loeffler recommends that as a prophylactic a gargle be used every three or four hours, consisting of a solution of bichloride of mercury of 1 to 15,000 to 1 to 10,000, or cyanide of mercury of 1 to 10,000 to 1 to 8,000. Chloroform-water is useful for the same purpose, and not unpleasant; and a 1 to 500 solution of thymol in twenty-per-cent alcohol.

In handling those sick of the disease he suggests using one of the weak gargles every one or two hours, and a 1 to 1,000 solution of sub-

limite; a three-per-cent solution of carbolic acid in thirty-per-cent alcohol, or a mixture of alcohol and turpentine, equal parts, containing two per cent of carbolic acid, every three or four hours. Finally, pencilling the throat with a five-per-cent solution of carbolic acid is added.

These solutions have been proven experimentally, not only to prevent the settlement and development of the bacilli on the adjacent healthy mucous membrane, but to destroy the bacilli in the deeper layers of the culture  $\frac{1}{2}$  mm. thick. And in two clinics in Berlin, one of Dr. Mosler and the other of Dr. Strübing, in which the carbolic acid and sublimate solutions were used respectively, the most excellent results were obtained; and whereas by ordinary methods of treatment virulent bacilli were found in the throat after three weeks, when the above methods were followed they could not be found after a few days.

Hence the disease is not only shortened by this treatment, but the affected individual ceases to be a menace to others much earlier than he would otherwise be.

In conclusion, I wish to emphasize the fact that in the last decade, by the employment of modern methods of research, more light has

been thrown upon this disease than in more than a half century before since its description, and that there is probably no other disease, hardly excepting tuberculosis, that has been rendered so clear in its etiology and pathology, so amenable to prophylaxis, and so promising to treatment.

LOUISVILLE.



